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Dopamine/serotonin receptor ligands. Part 17: A cross-target SAR approach: Affinities of azecine-styled ligands for 5-HT$_{2A}$ versus D$_1$ and D$_2$ receptors ☆

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ABSTRACT

Dibenzo- and benzindolo-azecines represent a novel class of high-affinity dopamine receptor antagonists. To further characterize these drugs as potential neuroleptics, we selected a set of azecine derivatives and ring expanded homologues and we measured their antagonist activity at the 5-HT$_{2A}$ receptor in the porcine coronary artery. SARs found for the 5-HT$_{2A}$ receptor resemble those for the D$_1$ but not the D$_2$ receptor. The protein–ligand interactions were discussed with respect to the different binding pockets.

Modern atypical antipsychotics display lower affinity for the dopamine D$_2$ receptor than for serotonin 5-HT$_{2A}$ receptor; therefore, these drugs are thought to produce fewer extrapyramidal side effects. In order to improve the profile of antipsychotic drugs, compounds with affinities for different receptor subtypes are being increasingly favored over highly selective compounds.[2] Recent efforts focus on the development of multi-acting receptor-targeted antipsychotics (MARTA).[3,4] The development of such promiscuous drugs affords the identification of 'cross-target SARs' to recognize how molecular modifications influence the affinity for the different targets.[5]

Azecine derivatives represent a novel class of anti-dopaminergic drug candidates with preferential binding to the D$_1$ receptor family (D$_1$,D$_5$).[1,6] Some preliminary studies have shown that azecines may be able to exhibit antagonist effects at 5-HT$_{2A}$ receptors as well,[7–9] which may enhance antipsychotic efficacy.

Accordingly, we selected 13 azecine-styled compounds and 11-membered benzindolo- and dibenzo-homologues in order to conduct a cross-target SAR investigation with regard to antagonistic activities at the dopamine and serotonin binding sites. The influences of hydroxylation and methoxylation of the aromatic rings, as well as different ways of enlarging the central azecine ring and the influence of different residues at the basic nitrogen were identified.

The affinity data for the human-cloned dopamine D$_1$ and D$_{2L}$ receptor subtypes have been previously measured in radioligand displacement experiments using [^{3}H]-SCH 23390 as a radioligand for the D$_1$ receptor and [^{3}H]-spiperone for the D$_2$ receptor, respectively.[1,6,10–12] They are given in Table 1 as pK_i values. Additionally, the compounds have been characterized as antagonists or inverse agonists by applying an intracellular Ca^{2+} assay. A detailed protocol is described in Ref. 1.

The screening for the affinity at serotonin 5-HT$_{2A}$ receptors was performed by measuring the isometric contractile force in isolated rings of porcine coronary artery as previously described.[13] 5-HT-induced contractions were competitively antagonized by the selective 5-HT$_{2A}$ receptor antagonist ketanserin (pA_2 8.88 ± 0.03). The affinity of ketanserin argues for an involvement of 5-HT$_{2A}$ receptors in the contractile response to 5-HT in this tissue.

Table 1 gives an overview of the affinity data for the D$_1$, D$_{2L}$ and 5-HT$_{2A}$ receptors. All of the compounds were found to be antagonists at the 5-HT$_{2A}$ receptor as well and they exhibited nanomolar or even subnanomolar affinities for 5-HT$_{2A}$, with the exception of the nor-compound **2i**, which only showed a micromolar affinity (pA_2: 7.2). Surprisingly, all of the investigated structural variations (i.e., hydroxy- and methoxy-groups at the aromatic moieties, different ways of enlarging the central azecine ring and different residues at the basic nitrogen) influenced the affinity for D$_1$ in more or less the same manner as for the 5-HT$_{2A}$ receptor.

☆ See Ref. 1.

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Table 1
Affinities (pK_i) for dopamine D_1 and D_2 receptor subtypes were determined by radioligand binding experiments

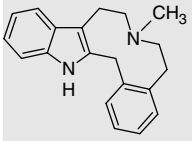
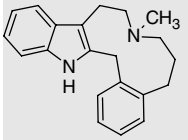
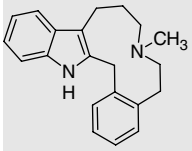
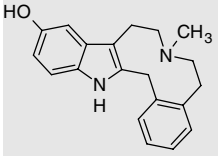
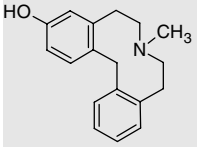
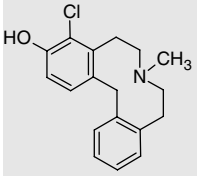
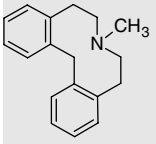
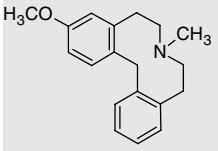
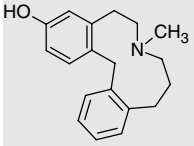
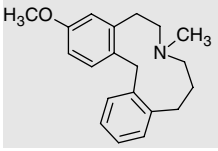
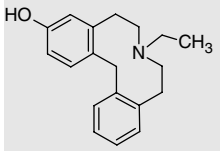
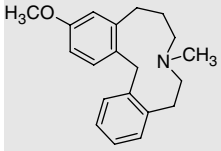
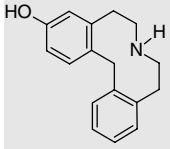
Compound		Affinity for D_1, nM ($pK_i \pm SEM$)[a]	Affinity for D_{2L}, nM ($pK_i \pm SEM$)[a]	Affinity for $5HT_{2A}$, ($pA_2 \pm SEM$)
	**1a(LE 300)**	8.73 ± 0.06	7.38 ± 0.16	9.79 ± 0.06[b]
	**1b**	8.69 ± 0.14	7.87 ± 0.13	9.77 ± 0.08[b]
	**1c**	6.80 ± 0.1	6.94 ± 0.45	8.42 ± 0.08
	**1d**	9.25 ± 0.05	7.36 ± 0.08	9.97 ± 0.06[b]
	**2a(LE404)**	9.45 ± 0.17	7.76 ± 0.04	9.19 ± 0.08
	**2b**	9.09 ± 0.06	8.45 ± 0.22	9.05 ± 0.09
	**2c**	8.46 ± 0.33	7.23 ± 0.1	8.70 ± 0.04
	**2d**	7.64 ± 0.1	7.89 ± 0	8.69 ± 0.07
	**2e**	8.574 ± 0.225	7.38 ± 0.42	8.67 ± 0.06
	**2f**	7.66 ± 0.20	7.062 ± 0.05	8.47 ± 0.05

Table 1 (*continued*)

Compound		Affinity for D_1, nM ($pK_i \pm SEM$)[a]	Affinity for D_{2L}, nM ($pK_i \pm SEM$)[a]	Affinity for $5HT_{2A}$, ($pA_2 \pm SEM$)
	**2g**	8.43 ± 0.09	7.11 ± 0.13	8.40 ± 0.02
	**2h**	7.55 ± 0.1	7.61 ± 0	8.24 ± 0.02
	**2i**	7.20 ± 0.13	6.41 ± 0.02	7.21 ± 0.06

[a] pK_i values are given for dopamine radioligand displacement experiments.
[b] pD_2' values are given for the experiments on porcine coronary artery.

This suggests that the binding cavities for this type of ligands at the D_1 and the 5-HT_{2A} receptors are similar. Such a correlation was not observed for the D_2 receptor. Therefore the following SAR discussion may refer to both the 5-HT_{2A} and the D_1 receptors.

Investigation of **1a**–**c** demonstrates the influence of ring enlargement in the benzindolo-series. Enlargement of **1a** yields in two 11-membered regioisomers. Formally, **1b** results from a replacement of the phenethylamine part with phenylpropylamine, whereas in **1c** the tryptamine moiety is replaced with homotryptamine. In the dibenzazecine-series, we compared the 10-membered **2d** to the respective 11-membered congeners **2f** and **h** (Fig. 1).

In general, expanding the ring to 11-membered heterocycles is tolerated but does not improve affinities. Remarkably, the affinities of the two regioisomeric 11-membered derivatives **2f** and **h** differ dramatically. The compounds with a tryptamine (**1b**) or a substituted phenethylamine moiety (**2f**) are superior to their three-carbon homologues **2h** and **1c** (Fig. 1).

To identify the influence of hydroxylation and methoxylation on the affinity, we compared the 10-membered phenolic **2a** and the respective methoxylated **2d**. In the ring enlarged compounds **2e** and **f**, the ring system is maintained and the only differences are determined by the substitution pattern (Fig. 2). The hydroxylated compounds **2a** and **e** generally display higher affinities than do their methoxylated counterparts **2d** and **f**.

The benefit of hydroxylation was also found in the benzindolo-series. Here the serotonin derivative **1d** is superior to the unsubstituted **1a**. (Fig. 2) Chlorination of **2a** gave **2b** and improved neither D_1 nor 5-HT_{2A} binding.

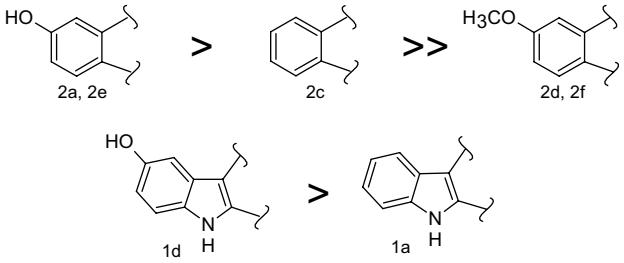


Figure 2. Influence of hydroxyl- and methoxy substituents on D_1 and 5-HT_{2A} binding.

Furthermore, we investigated the effect of the different residues –H, $-CH_3$ and $-C_2H_5$ at the basic nitrogen by comparing the 10-membered phenolic derivatives **2i** to **2a** and **2g**. Thereby, the N–C_2H_5 decreases the affinities significantly, but moreover an almost complete loss of affinity was achieved with the nor-compound (**2i**). Therefore, the N–CH_3 compounds were the most favorable, showing the highest affinities (Fig. 3). Any explanations regarding the low affinities of the secondary amine **2i** are speculative and should not be given now.

It is known that similar receptors bind similar drugs,[14] but from a phylogenetic point of view, the 5-HT_{2A} receptor and the D_1 receptor are not very similar.[15] Nevertheless only the structural properties of the binding cavities are responsible for protein–ligand interactions.

According to the findings of Surgand et al., we compared the 30 amino acids of the investigated receptors, which are supposed to shape the binding cavities of the respective receptors.[16] These ami-

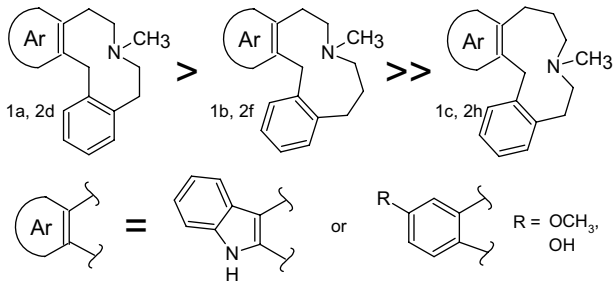


Figure 1. Influence of ring enlargement on D_1 and 5-HT_{2A} binding.

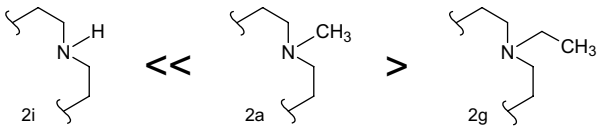


Figure 3. Influence of residues at the nitrogen on D_1 and 5-HT_{2A} binding.

Table 2
Comparison of amino acids, thought to line the binding cavities of the D1, D_2 and 5-HT_{2A} receptors, according to Surgand et al.

	1.35	1.39	1.42	1.46	2.57	2.58	2.61	2.65	3.28	3.29	3.32	3.33	3.36	3.40	4.56	4.60	5.38	5.39	5.42	5.43	5.46	6.44	6.48	6.51	6.52	6.55	7.35	7.39	7.43	7.45
D_2	Y	L	L	I	V	M	V	E	F	V	D	V	C	I	I	L	F	V	S	S	S	F	W	F	F	H	Y	I	Y	N
5-HT_{2A}	S	T	V	T	V	M	S	I	W	I	D	V	S	I	I	I	F	V	G	S	S	F	W	F	F	N	L	V	Y	S
D_1	T	L	L	T	V	M	K	E	W	V	D	I	S	I	I	P	Y	A	S	S	S	F	W	F	F	N	F	V	W	N
				X	X	X			X				X	X	X											X		X		

X: Amino acids, discussed for the resembling SARs at 5-HT_{2A} and D1 receptor.

no acids are given in Table 2 for the D_1, D_2, and 5-HT_{2A} receptors, and similarities are highlighted. Comparing the amino acids should allow the identification of critical residues that might explain our findings.

The following discussion not only refers to the relevant amino acids in Table 2, but also includes the cavities of 42 aminergic GPCRs, investigated by Surgand et al.

Within the proposed binding cavities, the D_1 receptor shares 16 identical amino acids with the 5-HT_{2A} receptor, whereas the D_2 receptor shares only 15 identical amino acids. The carboxylate residue of Asp D3.32, which is the counter ion to the protonated basic amine,[17] and the aromatic residues in TM6 (Phe F6.44/Trp W6.48/Phe F6.51/Phe F6.52) are crucial for a π–π-stacking with the aromatic moiety of biogenic amines and are highly conserved in all 42 aminergic GPCRs. Less common is the Asn N6.55 following the FWFF motif in TM6, which is only found in the 5-HT_4, 5-$HT_{2A,\ B,C}$, the adrenergic BAR1-3, and the D_1/D_5 receptors. The Val V7.39 occurs only at the 5-$HT_{2A,B,C}$, the D_1 and D_5 receptors and the trace amine receptor TAR03. Ser S3.36 followed by two Ileu I3.40 and I4.56 is within all 42 amine receptors found only in the D_1, D_5, and 5-HT_{2A} receptors. The Thr T1.46 followed by Val V2.51 and Met M2.58 is distributed only sparsely, namely in the D_1 family (D_1 and D_5), the 5-$HT_{1/2/5/6/7}$ receptors, the trace amine receptor TAR01, and the histamine H_1 receptor. Hence, the amino acids in 1.46, 2.57, 2.58, 3.28, 3.36, 3.40, 3.56, 6.55, and 7.39 might be responsible for our findings.

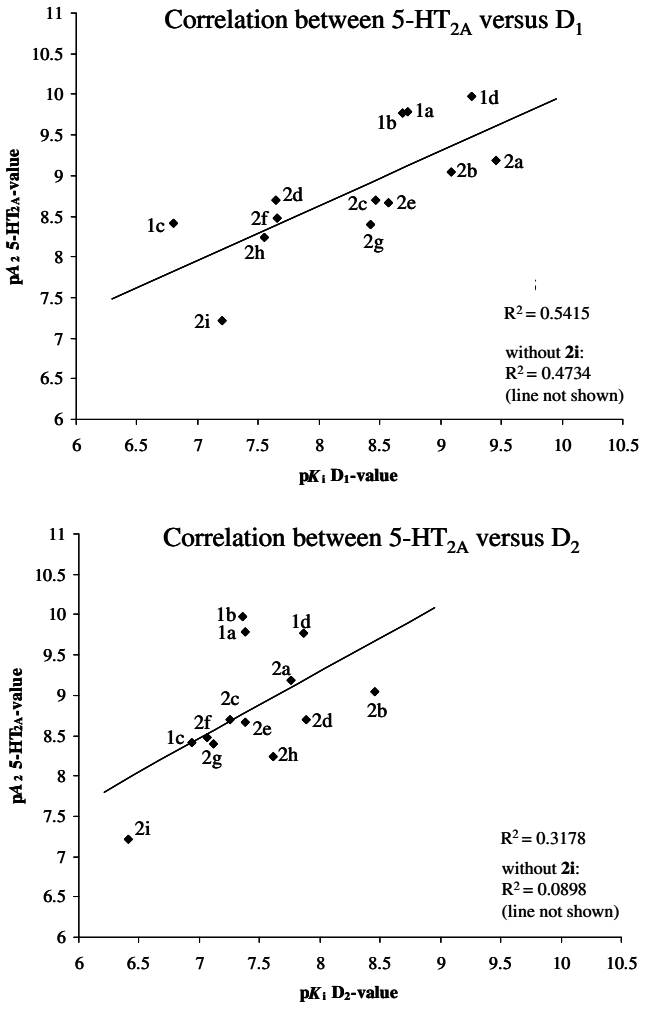


Figure 4. An overall correlation of the affinities for D_1, D_2 and 5-HT_{2A} receptors.

In view of the similar SARs, we found for the 5-HT_{2A} and D_1, but not the D_2 receptor, we put all of the pK_i-values in diagrams (Fig. 4). These diagrams illustrate a correlation of the affinities for 5-HT_{2A} and D_1 (left diagram) with a R^2 of 0.54 rather than for 5-HT_{2A} and D_2 with $R^2 = 0.31$ (right diagram). Since the secondary amine **2i** exhibits only micromolar affinities for all dopamine receptors, it makes the correlation between 5-HT_{2A} and D_2 look better than it is.

Omitting **2i** from both diagrams would leave the R^2-value of the correlation between 5-HT_{2A} and D_1 in the same range (0.47), whereas the R^2 of the correlation between 5-HT_{2A} and D_2 would drop significantly from 0.31 to 0.09.

Such consistency in the SARs for serotonin 5-HT_{2A} and dopamine D_1 has not been observed to our knowledge for other dopamine-serotonin ligands. Within the benzazepine-styled ligands with affinity for D_1 and 5-HT_{2A} receptors, such a comparison of SARs is difficult either due to the lack of data on the respective receptor subtypes[18,19] or because whether these compounds act and bind as agonists or antagonists is unknown.[20] Furthermore, for all of the D_1 receptor antagonists described previously, the affinities for D_1 are higher than those for 5-HT_{2A}, whereas for our azecine-styled compounds the 5-HT_{2A} affinities are generally higher than for D_1.

In conclusion, for azecine-styled compounds, ring size, aromatic substitution, and alkylation on the basic nitrogen affect the affinity for the 5-HT_{2A} receptor in the same manner than for D_1 but not for D_2 receptors.

These results are of great importance for the further understanding of binding mechanisms, but also for the development of azecine-like compounds as novel atypical antipsychotics. Especially since high and increasing affinities in the order $D_1 \cong D_2 < 5\text{-}HT_{2A}$ seem to be a promising feature for atypical neuroleptics.[3,4]

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